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### **Remarks/Arguments**

Applicants respectfully request favorable reconsideration of the subject application, particularly in view of the above amendment and the following remarks. Applicants respectfully urge that there is no additional fee for this amendment because the number of independent claims and the total number of claims have been reduced.

Applicants have amended Table 3 on page 23 of the subject application to provide required sequence ID numbers for each sequence set forth in the Table.

Applicants have amended Claim 1 of the subject application to define the detectable host characteristic as being growth rate whereby removal or deactivation of the characteristic gene defining the host growth rate results in a reduction of growth rate and reinsertion or reactivation of the characteristic gene substantially reestablishes the host growth rate defined by the characteristic gene. Applicants have similarly amended Claim 10. Support for this amendment may be found, for example, at page 14, lines 16-18 of the specification which indicates that *T. thermophilus* MM8-5 cells that possess expression vectors containing an *mdh* gene grow more rapidly than plasmid-free cells that lack a functional *mdh* gene. Accordingly, Applicants respectfully urge that this amendment is fully supported by the application as originally filed and, thus, incorporates no impermissible new subject matter into the application.

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Applicants have also canceled Claims 4, 6, 9 and 12-14 from the subject application.

Claims 1-14 are currently pending in the subject application of which Claims 1-13 have been rejected and Claim 14 has been withdrawn as being directed to a non-elected invention.

The Examiner has required restriction of the subject application to one of two inventions alleged to be disclosed and claimed in the subject application - Claims 1-13 drawn to a method for introducing and stabilizing heterologous and recombinant genes in a thermophilic host and Claim 14 drawn to an integrative vector. In response to this restriction requirement, a provisional election with traverse was made by the undersigned during a telephone conversation with the Examiner on 20 October 2003 to prosecute the invention of Claims 1-13. Applicants hereby affirm this election. As a result of this election, Applicants have canceled Claim 14 from the subject application.

The Examiner has indicated that the application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR §1.821(a)(1) and (a)(2) but fails to comply with the requirements of 37 CFR §§ 1.821-1.825 for the reasons set forth in the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence

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and/or Amino Acid Sequence Disclosures enclosed with the Office Action. In particular, the Examiner indicates that there are sequences listed in Table 3 of the application that do not appear in the Sequence Listing. In response, Applicants have amended Table 3 of the application to provide proper sequence identification numbers for each listed sequence. Applicants have also amended the Sequence Listing to include these sequences. Accordingly, Applicants are enclosing with this amendment 1) a substitute computer readable form (CRF) copy of the Sequence Listing, 2) a substitute paper copy of the Sequence Listing, and 3) a copy of the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures. Applicants respectfully urge that the content of the paper and computer readable copies of the Sequence Listing are the same and include no new matter.

The invention claimed by Applicants is a method for introducing and stabilizing heterologous and recombinant genes in a thermophilic host in which a characteristic gene in the thermophilic host which defines host growth rate is inactivated or removed from the thermophilic host. The result is a modified thermophilic host having a reduced growth rate. A DNA fragment of interest is then inserted into the modified thermophilic host together with an intact or restored characteristic gene, as a result of which the host growth rate increases, thereby

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enabling detection or confirmation of successful transformation using plasmid vectors and integration of the DNA fragment into a chromosome of the thermophilic host. In accordance with one embodiment of the claimed invention, the detectable characteristic gene is the malate dehydrogenase gene (*mdh*), which, when rendered inactive in the *Thermus* sp., results in a low growth rate phenotype, i.e. a “sick” colony phenotype. The initial higher growth rate phenotype, i.e. “healthy” phenotype, is restored by introduction/transformation of a plasmid, integration vector or DNA fragment containing an intact *mdh* gene as well as a gene of interest. In this manner the presence of the gene of interest in the thermophilic host can be confirmed. Applicants respectfully urge that the prior art relied upon by the Examiner as the basis for rejection of the subject application neither teaches nor suggests a method in which a gene defining growth rate of a thermophilic host is used to confirm the successful insertion of a gene of interest into the thermophilic host based upon the modification and subsequent restoration of growth rate through deactivation or removal followed by reactivation or restoration of the gene as claimed by Applicants.

Claims 1 and 7 have been rejected under 35 U.S.C. 102(b) as being anticipated by Knol et al., U.S. Patent 5,491,079 (hereinafter “the Knol et al. patent”). This rejection is respectfully traversed. The Knol et al. patent teaches a method for gene integration into the lac operon of *Streptococcus Thermophilus* in which a portion

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of the *lacZ* gene in the host strain is deleted, imparting a lac(-) phenotype to the host strain, and a donor plasmid comprising a vector backbone and a foreign gene as well as the fragment deleted from the *lacZ* gene of the host strain is integrated into the genomic lac operon of the host strain, thereby restoring the *lacZ* gene and enabling its expression under selective pressure. However, the Knol et al. patent neither teaches nor suggests a method as claimed by Applicants in which the characteristic gene is one, such as the *mdh* gene, which affects the growth rate of the thermophilic host, such that the absence thereof results in a low growth rate ("sick") phenotype and restoration thereof in combination with insertion of a gene of interest restores the growth rate of the thermophilic host, resulting in a higher growth rate ("healthy") phenotype and confirmation of the insertion of the gene of interest. Accordingly, Applicants respectfully urge that the Knol et al. patent neither teaches nor suggests the method of the invention claimed by Applicants and, thus, does not anticipate the invention claimed by Applicants in the manner required by 35 U.S.C. 102(b).

Claims 1-3 and 5 have been rejected under 35 U.S.C. 102(a) as being anticipated by Kayser et al., J. Bacteriol., Vol. 183, No. 5, pp. 1792-1795 (Mar. 2001) (hereinafter "the Kayser et al. reference"). This rejection is respectfully traversed. The subject application has a filing date of 28 February 2002, which filing date is less than one year following the date of publication of the Kayser et al. reference. In

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addition, all of the authors of the Kayser et al. reference are named inventors on the subject application. Applicants respectfully urge that, because the Kayser et al. reference has a publication date less than one year preceding the filing date of the subject application, and because all of the authors of the Kayser et al. reference are inventors of the invention claimed in the subject application, the Kayser et al. reference is not prior art properly cited against the subject application. MPEP § 706.02(a)III states that, for 35 U.S.C. 102(a) to apply, the reference must have a publication date earlier in time than the effective filing date of the application, *and must not be applicant's own work*. Because the cited reference is Applicants' own work, and because the reference has a publication date less than one year prior to the effective filing date of the subject application, Applicants respectfully urge that the Kayser et al. reference does not anticipate the invention claimed by Applicants in the manner required by 35 U.S.C. 102(a).

Claims 1, 3, 5 and 10 have been rejected under 35 U.S.C. 102(b) as being anticipated by Tamakoshi et al., J. Bacteriol., Vol. 179, No. 15, pp. 4811-4814 (hereinafter "the Tamakoshi et al. reference"). This rejection is respectfully traversed. The Tamakoshi et al. reference teaches a *Thermus thermophilus* strain in which the *pyrE* gene is deleted, rendering the host unable to synthesize uracil and to become immune to the effects of the uracil precursor analogue, 5-fluorotic acid. When the

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*T. thermophilus*  $\Delta$ *pyrE* culture is grown on nutritionally rich media, good growth results, in direct contrast to the invention claimed by Applicants in which the characteristic gene, e.g. *mdh*, when inactivated or removed from the thermophilic host, renders the culture metabolically “sick”. Accordingly, Applicants respectfully urge that the Tamakoshi et al. reference, which neither teaches nor suggests a method for introducing and stabilizing heterologous and recombinant genes in a thermophilic host employing a characteristic gene in the thermophilic host which defines host growth rate as the basis for confirming insertion of the gene of interest as claimed by Applicants, does not anticipate the invention claimed by Applicants in the manner required by 35 U.S.C. 102(b).

Claims 1 and 7 have been rejected under 35 U.S.C. 102(b) as being anticipated by Mollet et al., J. Bacteriol., Vol. 175, No. 14, pp 4315-4324, (hereinafter “the Mollet et al. reference”). This rejection is respectfully traversed. The Mollet et al. reference teaches the use of *lacZ* genes in genetic studies with *Streptococcus thermophilus*. Although useful as a genetic marker that can be easily detected, nowhere does the Mollet et al. reference teach or suggest that inactivation or removal of the *lacZ* gene results in a lower growth rate culture as occurs with the *mdh* gene in accordance with the method of the invention claimed by Applicants. Accordingly,



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Applicants respectfully urge that the Mollet et al. reference does not anticipate the invention claimed by Applicants in the manner required by 35 U.S.C. 102(b).

Claims 1, 3, 5, 8, 10 and 13 have been rejected under 35 U.S.C. 103(a) as being unpatentable over the Tamakoshi et al. reference discussed herein above in view of Peredultchuk et al., U.S. Patent 6,344,327 (hereinafter “the Peredultchuk et al. patent”). This rejection is respectfully traversed. Applicants’ arguments with respect to the Tamakoshi et al. reference as set forth herein above are equally applicable to this rejection and, thus, will not be repeated. The Peredultchuk et al. patent is relied upon by the Examiner for its teachings regarding the use of the beta-galactosidase gene as a selective marker. By way of the above amendment, Applicants have canceled those claims directed to the use of beta-galactosidase genes; accordingly, Applicants respectfully urge that this rejection is rendered moot.

Claims 1, 3, 5, 10 and 11 have been rejected under 35 U.S.C. 103(a) as being unpatentable over the Tamakoshi et al. reference in view of Nishiyama et al., J. Biol. Chem., 261 (30), pp. 14178-14183, (hereinafter “the Nishiyama et al. reference”). This rejection is respectfully traversed. Applicants’ arguments with respect to the Tamakoshi et al. reference as set forth herein above are equally applicable to this rejection and, thus, will not be repeated other than to reiterate that the Tamakoshi et al. reference neither teaches nor suggests the use of genes affecting



the growth rate of a thermophilic strain as the basis for integration of a gene of interest into the thermophilic strain as required by the method of the invention claimed by Applicants. The Nishiyama et al. reference discloses the nucleotide sequence of the malate dehydrogenase (*mdh*) gene from a thermophilic bacterium as well as mutation of the *mdh* gene to produce an increase in enzyme activity. However, the Nishiyama et al. reference neither teaches nor suggests the inactivation or deletion of the *mdh* gene to create metabolically crippled hosts, that is hosts having a reduced growth rate, nor does it teach or suggest the use of vectors containing an intact *mdh* gene to restore the hosts to a metabolically healthy condition. Accordingly, Applicants respectfully urge that the Tamakoshi et al. reference and the Nishiyama et al. reference, alone or in combination, do not render Applicants' claimed invention obvious in the manner required by 35 U.S.C. 103(a).

In summary, none of the prior art references relied upon by the Examiner as the basis for rejection of the subject application teach or suggest usage of a characteristic gene in the manner of the invention claimed by Applicants. Neither the *lacZ* gene nor the *pyrE* gene disclosed by the cited prior art can be used to select for the survival of transformants (genes of interest) by growing the culture on nutritionally rich medium. However, when  $\Delta mdh$  hosts in accordance with one embodiment of the method of the invention claimed by Applicants are transformed

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with *mdh*-containing vectors, then only the *mdh*-containing transformants are positively selected due to the faster growth rates on nutritionally rich media. In contrast thereto, the *lacZ*<sup>+</sup>,  $\Delta$ *lacZ*, *pyrE* wild type and  $\Delta$ *pyrE* cultures will all have the same growth rate in nutritionally rich media. Thus, growth in nutritionally rich media cannot be used to select for *lacZ*-containing transformants of  $\Delta$ *lacZ* hosts or for *pyrE*-containing transformants of  $\Delta$ *pyrE* hosts.

Claims 4, 6, 9 and 12 have been rejected under 35 U.S.C. 112, first paragraph as failing to comply with the enablement requirement. Applicants respectfully urge that, as a result of the cancellation of these claims from the subject application, this rejection is rendered moot.

Claims 1-13 have been rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Examiner indicates that Claim 1 and Claim 10 and by dependence Claims 2-9 and 11-13 are vague and indefinite in the recitation of “one of activating and deleting” and “one of detection and confirmation”, “detection or confirmation” and “one of an inactivated and deleted characteristic gene” because it is not clear what is intended by these phrases. In response thereto, Applicants have amended Claims 1 and 10 to delete such phrases and substitute therefor the phrases “inactivating or deleting” and “an inactivated or

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a deleted characteristic gene”. Applicants respectfully urge that this amendment overcomes this rejection.

Claim 1 and, by dependence, Claims 2-9 have been indicated to be vague and indefinite in the recitation of “detection and confirmation of successful transformation using plasmid vectors and integration of said DNA fragment into a chromosome of said thermophilic host”. In response to this rejection, Applicants have amended Claim 1 by modifying this recitation to read “detection or confirmation of successful transformation using plasmid vectors or integration of said DNA fragment into a chromosome of said thermophilic host.” Applicants respectfully urge that this amendment overcomes this rejection.

Claim 6 has been indicated to be vague and indefinite in its recitation of “CARD mutant strain”. In response thereto, Applicants have canceled Claim 6, thereby rendering this rejection moot.

The use of the term “strong” in Claim 10 has been objected to as being indefinite as it is a relative term for which no distinct definition has been provided. In response thereto, Applicants have deleted this term from the claim. Accordingly, Applicants respectfully urge that this amendment overcomes this rejection.

Finally, Claim 10 has been indicated to be vague and indefinite in its recitation of the transforming step before the cloning step. In response thereto,

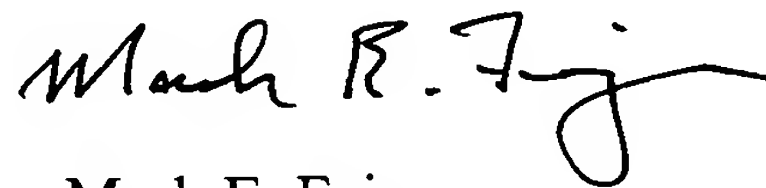
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Applicants have amended Claim 10 to reverse the order of these steps. Applicants respectfully urge that this amendment overcomes this rejection.

Applicants intend to be fully responsive to the outstanding Office Action. If the Examiner detects any issue which the Examiner believes Applicants have not addressed in this response, Applicants urge the Examiner to contact the undersigned.

Applicants sincerely believe that this patent application is now in condition for allowance and, thus, respectfully request early allowance.

Respectfully submitted,



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